A flow chart for array-based detection of gene expression
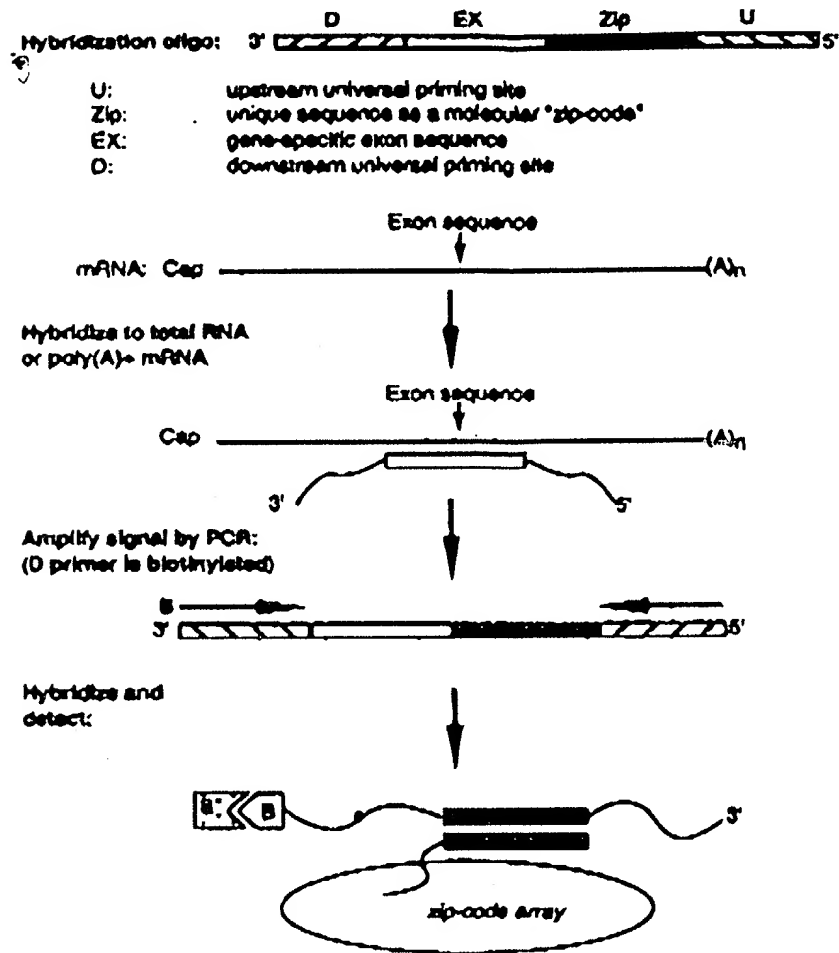
Hybridization oligo:

	D	EX	Zip	U	
3'					5'

- U: upstream universal priming site
- Zip: unique sequence as a molecular "zip-code"
- EX: gene-specific exon sequence
- D: downstream universal priming site

Exon sequence

mRNA: Cap ———————————— $(A)_n$

Hybridize to total RNA
or poly(A)+ mRNA

Exon sequence

Cap ———————————— $(A)_n$

3'   5'

Amplify signal by PCR:
(D primer is biotinylated)

3' ———————————— 5'

Hybridize and
detect:

zip-code array

Figure 1

A flow chart for array-based detection of RNA alternative splicing

Hybridization oligo: 3' D   SJ   Zip   U 5'

U: upstream universal priming site
Zip: unique sequence as a molecular "zip-code"
SJ: gene-specific splice junction
D: downstream universal priming site

Splice junction
mRNA: Cap —————————————————————————(A)n

Hybridize to total RNA
or poly(A)+ mRNA

Splice junction
Cap —————————————————————————(A)n

3'   5'

Amplify signal by PCR:
(D primer is biotinylated)

3'   5'

Hybridize and
detect:

3'

zip-code array
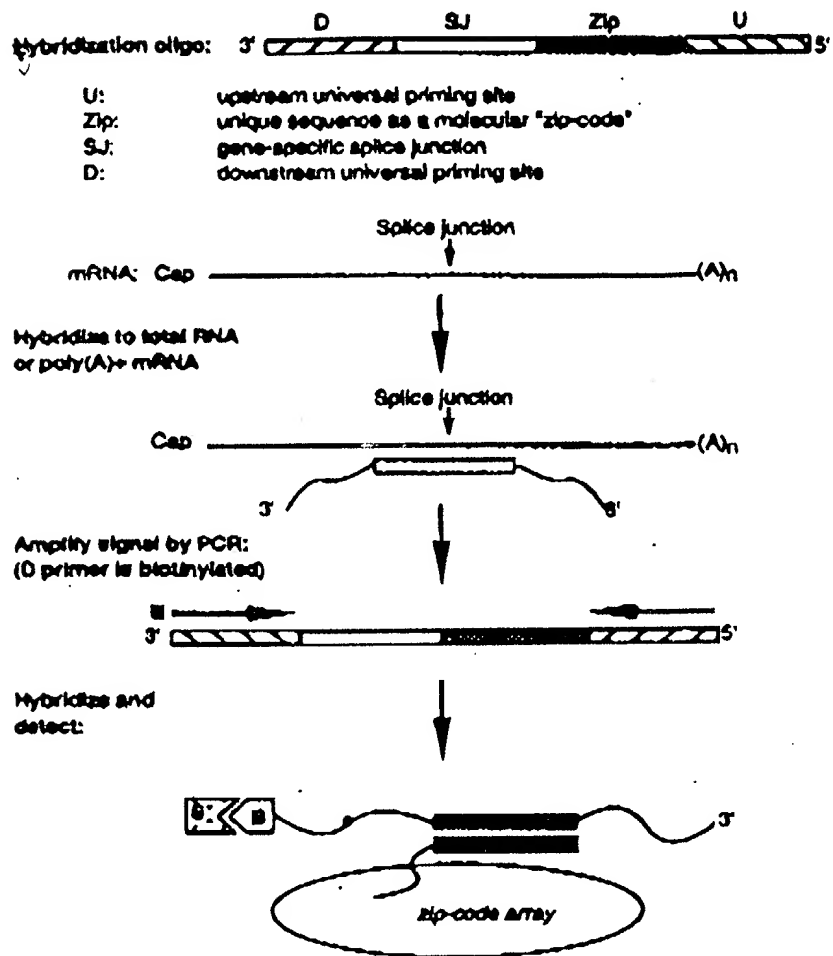
Figure 2

Genome-wide gene expression profiling using oligo-ligation strategy
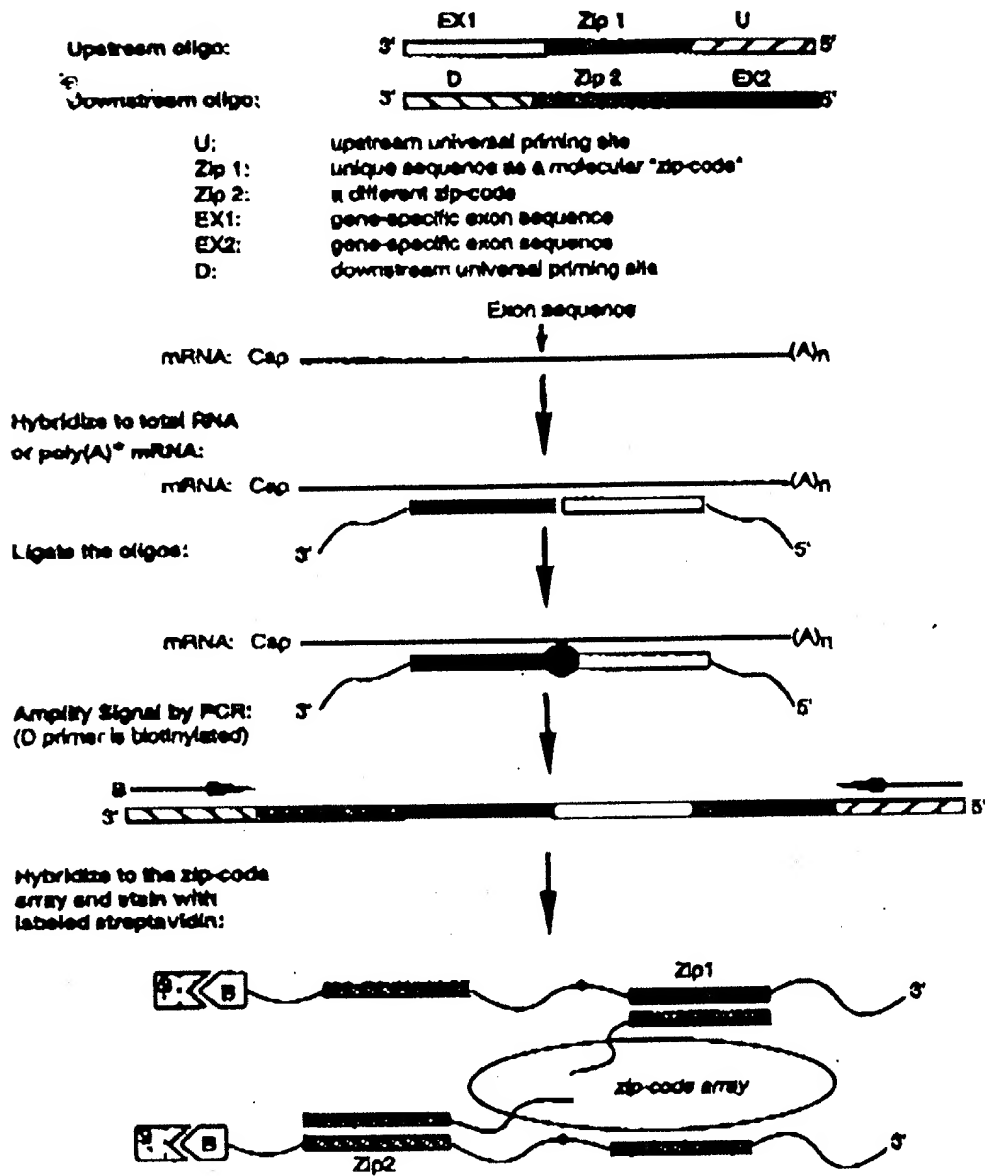
Figure 3

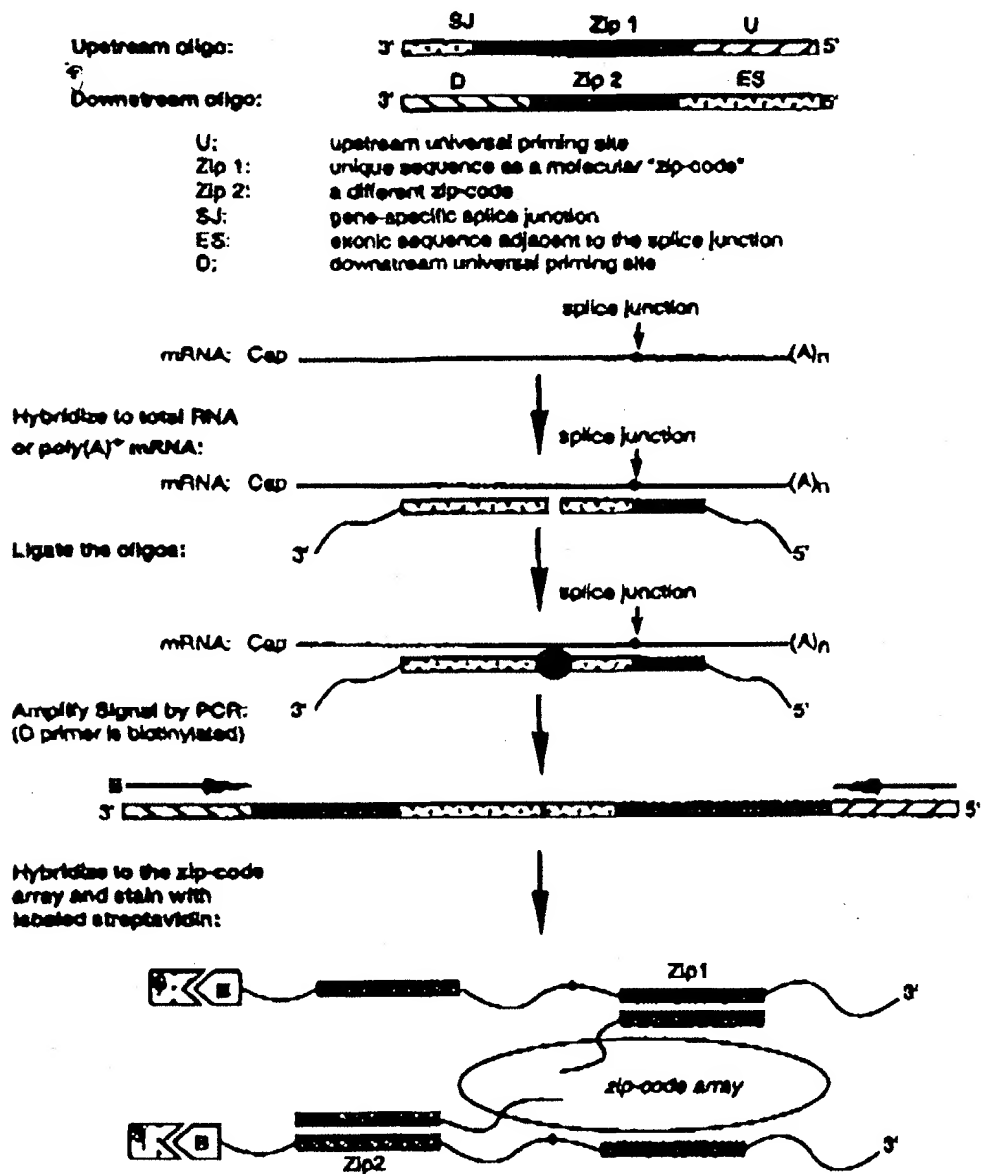Genome-wide RNA alternative splicing monitoring using oligo-ligation strategy

Upstream oligo:
   SJ   Zip 1   U

Downstream oligo:
   D   Zip 2   ES

U: upstream universal priming site
Zip 1:   unique sequence as a molecular "zip-code"
Zip 2:   a different zip-code
SJ: gene-specific splice junction
ES: exonic sequence adjacent to the splice junction
D: downstream universal priming site

splice junction

mRNA: Cap _____ (A)n

Hybridize to total RNA
or poly(A)+ mRNA:

splice junction

mRNA: Cap _____ (A)n

Ligate the oligos:

splice junction

mRNA: Cap _____ (A)n

Amplify Signal by PCR:
(D primer is biotinylated)

Hybridize to the zip-code
array and stain with
labeled streptavidin:

Zip1

zip-code array

Zip2

Figure 4

Upstream oligo:

SNP_A   Zip 1   U

3'   5'

Downstream oligo:

D   Zip 2   SNP_B

3'   5'

U:	upstream universal priming site
Zip 1:	unique sequence as a molecular "zip-code"
Zip 2:	a different zip-code
SNP_A:	SNP-specific sequence
SNP_B:	SNP-specific sequence
D:	downstream universal priming site

Polymorphic site

genomic DNA:

Hybridize to genomic DNA:

genomic DNA:

Ligate the oligos:

3'   5'

genomic DNA:

Amplify signal by PCR:
(D primer is biotinylated)

3'   5'

B   5'

3'   5'

Hybridize to the zip-codes
array and stain with
labeled streptavidin:

Zip1   3'

zip-code array

Zip2   3'

Figure 5

Upstream oligo: 3' [Target_U | Zip 1 | U] 5'

Downstream oligo: 3' [D | Zip 2 | Target_D] 5'

Middle oligo: 3' [Target_M] 5'

U: upstream universal priming site
Zip 1: unique sequence as a molecular "zip-code"
Zip 2: a different zip-code
Target_U: upstream target-specific sequence
Target_D: downstream target-specific sequence
Target_M: middle target-specific sequence
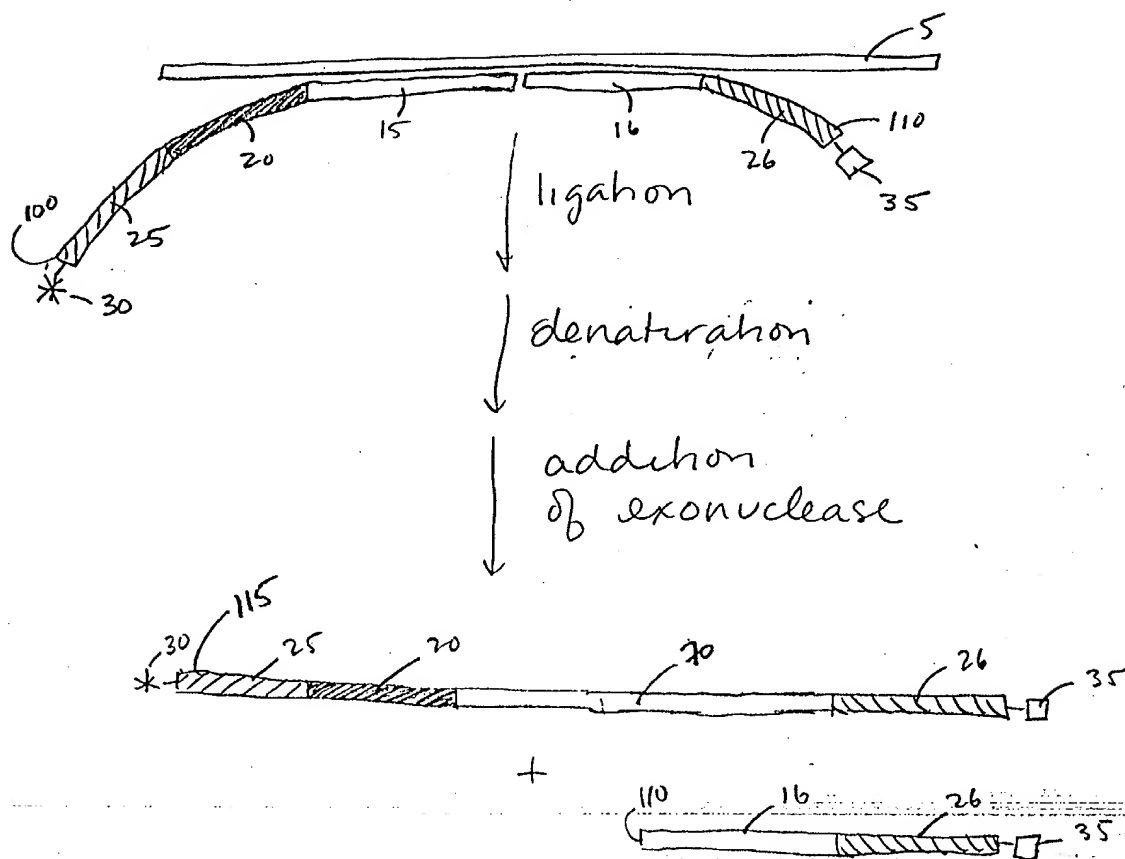D: downstream universal priming site

Target: ——————————————

Hybridize to target:

Target:

Ligate the oligos: 3'   5'

Target:

Amplify Signal by PCR: 3'
(D primer is biotinylated)

B —>
T   5'

Hybridize to the zip-code
array and stain with
labeled streptavidin:

Zip1

zip-code array

Zip2

Figure 6

unhybridize
probes

removal/wash
of unhybridized
probes

denature

amplify

Fig 7

ligation

denaturation

addition of exonuclease
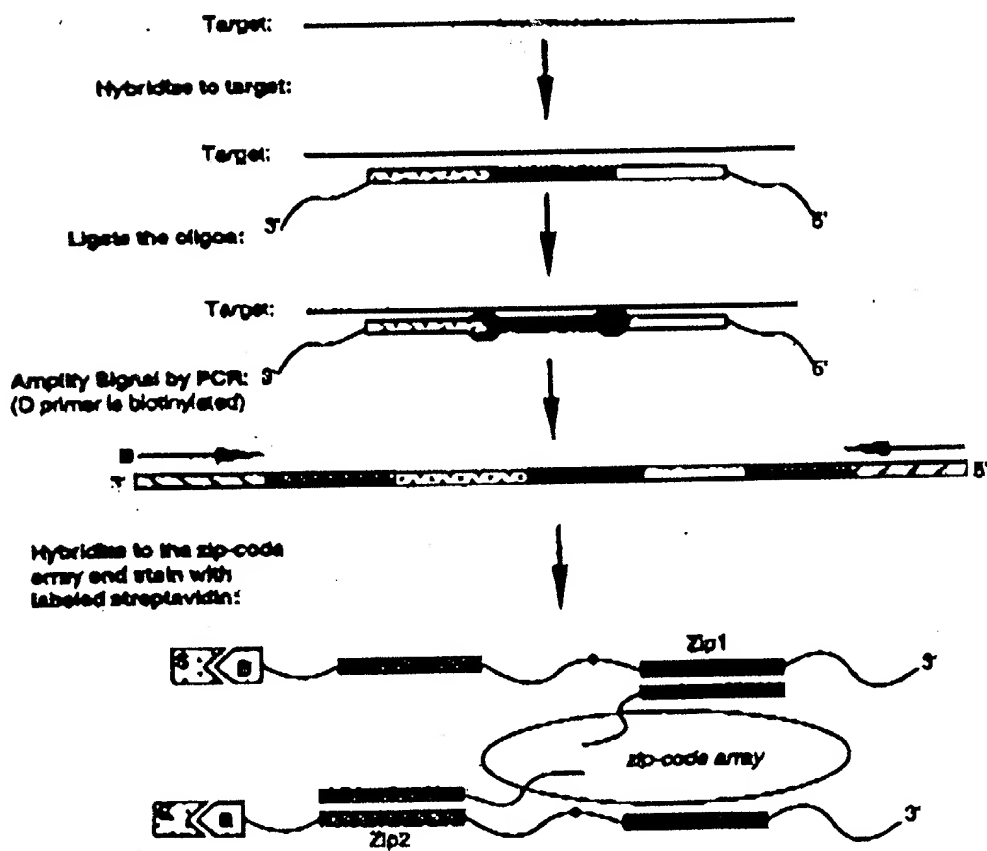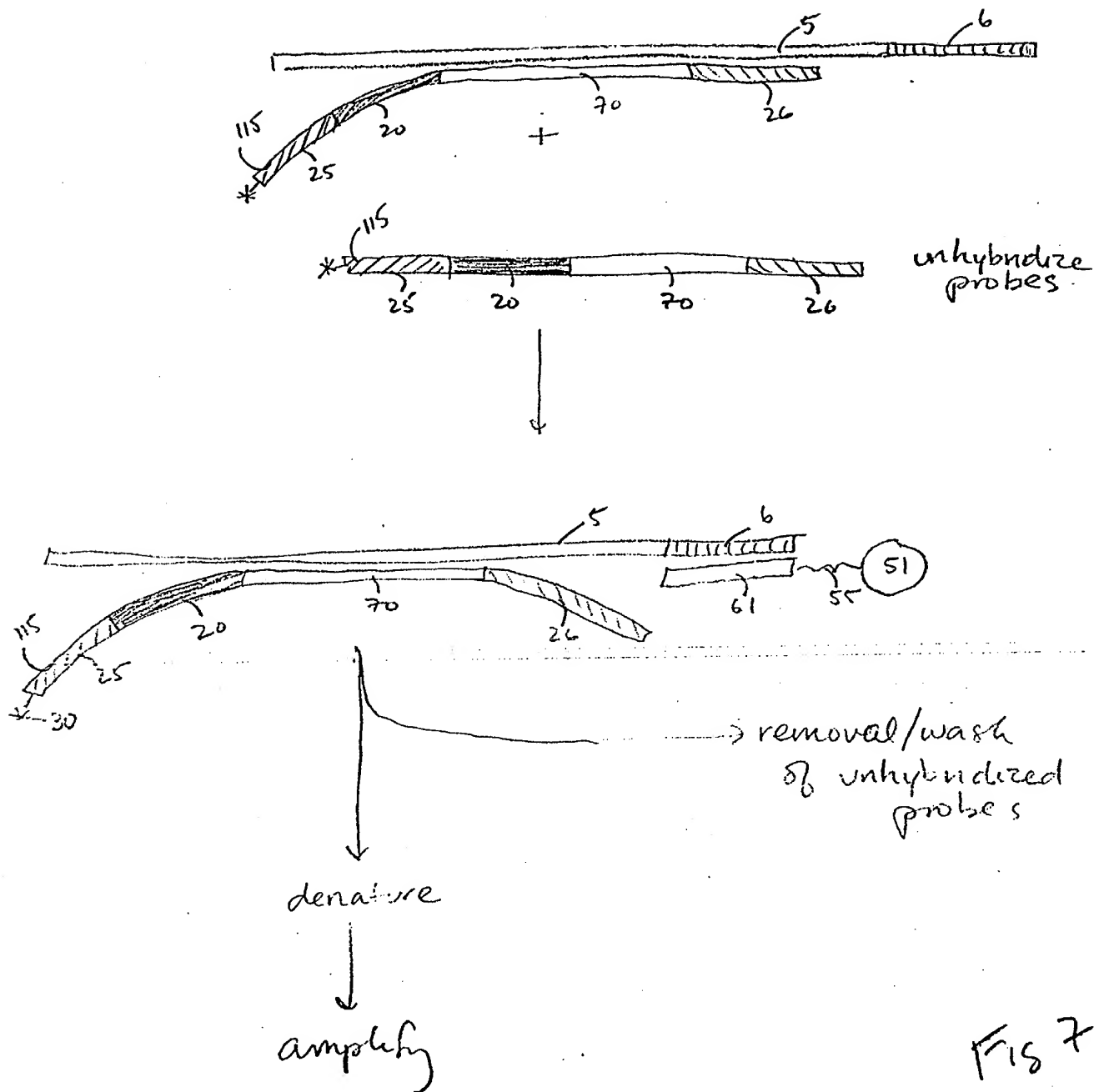
addition to array, wash away unbound

FIG 8

ligation

ligation
polymerase

FIG 9

5

140   15   16

20   150

| ligation, denaturation

70

140

20   15
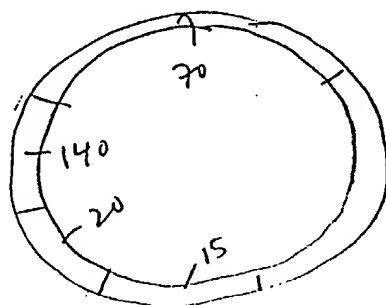
| addition of
primer,
extension

Fig 10